Federal Democratic Republic of Ethiopia

EDICT OF GOVERNMENT

In order to promote public education and public safety, equal justice for all, a better informed citizenry, the rule of law, world trade and world peace, this legal document is hereby made available on a noncommercial basis, as it is the right of all humans to know and speak the laws that govern them.

ET ISO 8128-2 (2012) (English): Apple juice, apple juice concentrates and drinks containing apple juice - Determination of patulin content, Part 2: Method using a thin layer chromatography

ISO INSIDE

BLANK PAGE

BRUTUM FULMEN

IGNORANTIA JURIS NON EXCUSAT

PROTECTED BY COPYRIGHT

ETHIOPIAN
STANDARD

ES ISO 8128-2:2012

First edition

Apple juice, apple juice concentrates and drinks containing apple juice - Determination of patulin content - ~~Part 1: Method using a thin-layer chromatography~~

Part 2: Method using a thin layer chromatography

ICS: 67.160.20

Published by Ethiopian Standards Agency

©ESA

Foreword

This Ethiopian Standard has been prepared under the direction of the Technical Committee for Non Alcoholic Beverages (TC 27) and published by the Ethiopian Standards Agency (ESA). The standard is identical with ISO 8128-2:1993 Apple juice, apple juice concentrates and drinks containing apple juice - Determination of patulin content - Part 2: Method using a thin-layer chromatography published by the International Organization for Standardization, ISO 1993.

For the purpose of this Ethiopian Standard, the adopted text shall be modified as follows.

- The phrase “International Standard” shall be read as “Ethiopian Standard”; and
- A full stop (.) shall substitute comma (,) as decimal marker.

Apple juice, apple juice concentrates and drinks containing apple juice — Determination of patulin content —

Part 2:
Method using thin-layer chromatography

1 Scope

This part of ISO 8128 specifies a method using thin-layer chromatography for the determination of the patulin content of apple juice, apple juice concentrates and drinks containing apple juice.

The limit of detection of the method is 25 µg/l, based on 50 ml of ready-to-drink apple juice.

NOTE 1 For more precise analyses or in case of a dispute, the HPLC method specified in ISO 8128-1 should be used.

2 Principle

Extraction of patulin in a mixture of ethyl acetate and chloroform (3:2 by volume). Filtration of the extract on a silica-gel column and qualitative and semi-quantitative determination by means of two-directional thin-layer chromatography (TLC). The spots are developed using a 3-methyl-2-benzothiazoline hydrazone (MBTH) hydrochloride solution.

3 Reagents

Use only reagents of recognized analytical grade and water of the purity required for chromatography.

WARNING — Special attention should be paid when using benzene or chloroform, which are toxic and may cause explosions.

3.1 Solvents, ethyl acetate, chloroform and toluene.

3.2 Developing solvents, for two-directional TLC:

benzene/methanol/acetic acid (80 % by mass) mixture (19:2:1 by volume);

toluene/ethyl acetate/formic acid (90 % by mass) mixture (5:4:1 by volume).

3.3 Silica gel, for column chromatography, of 0,063 mm to 0,2 mm particle size.

3.4 Eluting solution, toluene/ethyl acetate mixture (75:25 by volume).

3.5 Patulin standard solution ($C_7H_6O_4$).

3.5.1 Preparation

Weigh, to the nearest 0,1 mg, 10,0 mg of patulin in a 100 ml one-mark volumetric flask and dissolve it in ethyl acetate (3.1). Make up to the mark with ethyl acetate.

Pipette 10,0 ml of this solution into another 100 ml one-mark volumetric flask and make up to the mark with ethyl acetate.

The patulin content of this standard solution is 10 µg/ml approximately.

Measure the absorbance at 276 nm of this standard solution on an appropriate spectrometer using quartz cells of optical path length 10 mm.

NOTE 2 The preparation of the standard solution and the control of its purity are based on reference [3].

©ESA

3.5.2 Calculation of the concentration

Calculate the concentration ρ_{ps}, expressed in micrograms per millilitre, of the patulin solution (3.5.1) using the formula

$$\rho_{ps} = \frac{A \times M_r \times 1\,000 \times C}{A_{276}}$$

where

A is the absorbance of the patulin standard solution;

A_{276} is the molecular absorbance of the patulin solution at the maximum (276 nm) of the absorption spectrum (see note 3).

M_r is the relative molecular mass of patulin;

C is the apparatus constant (usually 1).

NOTE 3 The molecular absorbance coefficient of patulin measured in ethanol at 276 nm is equal to 14 600.

3.6 MBTH hydrochloride solution

Dissolve 0,5 g of 3-methyl-2-benzothiazoline hydrazone (MBTH) hydrochloride monohydrate in 100 ml of water.

Store in a refrigerator and prepare a fresh solution at least every 3 days.

4 Apparatus

Rinse the laboratory apparatus before use with a 10 g/l sodium hypochlorite solution.

Usual laboratory apparatus and, in particular, the following.

4.1 Chromatographic columns, of dimensions 300 mm × 22 mm, with a 250 cm^3 reservoir and a stopcock, equipped at one end with a sintered glass disc.

4.2 TLC equipment, glass developing tanks, long-wavelength ultraviolet (UV) lamp (360 nm) and spraying device.

4.3 Fluorodensitometer

4.4 TLC plates, 20 cm × 20 cm, coated with silica gel (3.3) (layer thickness 0,25 mm), without a fluorescent indicator.

4.5 Oven, ventilated, capable of operating at 130 °C ± 1 °C.

5 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

6 Procedure

6.1 Preparation of test solution

Dilute apple juice concentrates with water, using a 1:5 mixture of concentrate to water, by volume. Then proceed as for the other products, as follows.

Extract 50 ml of the laboratory sample (diluted with water if necessary) with a 50 ml portion of ethyl acetate/chloroform mixture (3:2 by volume) for at least 1 min.

Repeat the extraction with two more 50 ml portions of ethyl acetate/chloroform mixture and filter each portion through a sintered glass funnel, containing a 1 cm layer of anhydrous sodium sulfate, collecting the filtrate directly in a 250 ml evaporation flask.

Evaporate to dryness under vacuum using a rotary evaporator and transfer quantitatively the residue thus obtained into a 100 ml graduated cylinder, rinsing the flask with four 5 ml portions of ethyl acetate. Dilute to 25 ml with ethyl acetate, and then to 100 ml with toluene.

6.2 Column chromatography

Place a small plug of glass wool in the bottom of the column (4.1), then add 25 ml of toluene. Add a slurry comprising 15 g of silica gel (3.3) in 40 ml of toluene to the column, and then add 15 g of anhydrous sodium sulfate and drain off the solvent until the surface of the solvent is level with the top of the packing. Add the extract of the test portion to the column and allow to drain until its surface is level with the top of the packing. Discard the eluate. Add 200 ml of eluting solution (3.4) to the column and collect the eluate in a graduated cylinder, using a flow rate of about 5 ml/min. Evaporate the eluate until about 2 ml remains.

Transfer quantitatively the concentrated eluate to a 20 ml tube, using four portions (of about 4 ml each) of ethyl acetate and evaporate to dryness under a stream of nitrogen. Immediately dissolve the residue in 500 µl of ethyl acetate since patulin is not stable.

6.3 Thin-layer chromatography

Activate the pre-coated TLC plates (4.4) at 110 °C for 2 h. Spot, using a capillary pipette or microsyringe, a 20 µl aliquot portion of the purified extract of the sample (6.2) onto the TLC plate at a distance of 2 cm from its left-hand edge and 2 cm from its lower edge. Spot a 5 µl aliquot portion of the patulin standard solution (3.5) onto the TLC plate at a distance of 2 cm from its right-hand edge and 2 cm from its lower edge. Spot a 5 µl aliquot portion of the patulin standard solution 2 cm from the left-hand edge and 2 cm from the upper edge. Spot a 10 µl aliquot portion of the patulin standard solution 2 cm from the left-hand edge and 4 cm from the upper edge (see figure 1).

Develop the chromatogram in direction I using the benzene/methanol/acetic acid mixture (3.2) until the solvent front reaches a distance of 14 cm. Remove the plate from the tank, dry in air and then develop the chromatogram in direction II using the toluene/ethyl acetate/formic acid mixture (3.2) until the solvent front reaches a distance of 14 cm. Remove the plate from the tank and allow to dry at ambient temperature. Spray the TLC plate with the MBTH hydrochloride solution (3.6) and then dry for 15 min in the oven (4.5) set at 130 °C.

6.4 Determination

Determine the quantity of patulin in the extract by comparing the intensity of fluorescence of the yellow-brownish spot from the extract with those of the standard reference spots under UV long-wavelength irradiation. Look for the patulin spot from the extract at the intersection of the perpendicular lines originating from the standard reference spots. If the intensity of fluorescence given by the 20 µl of extract is greater than those of the standard reference spots, dilute the extract with ethyl acetate and repeat the TLC procedure specified in 6.3. If the intensity of fluorescence of the spot from the extract is comparable with one of those of the standard reference spots, it means that the concentration of patulin in the sample analysed is either 25 µg/l or 50 µg/l respectively.

NOTE 4 When using the method specified, the separation of patulin from HMF[1] is complete, and therefore there is no risk of interference.

7 Calculation

Calculate the patulin content of the sample, ρ_p, in micrograms per litre, as follows:

$$\rho_p = \frac{V_2 \times \rho_{ps} \times V_0}{50V_1}$$

where

V_0 is the volume, in microlitres, of the final dilution of the purified extract of the test portion;

V_1 is the volume, in microlitres, of the spot of the test portion;

V_2 is the volume, in microlitres, of the spots of the patulin standard solution (3.5) giving an intensity of fluorescence equal to that given by the spot of the test solution;

ρ_{ps} is the concentration, in micrograms per millilitre, of the patulin standard solution.

8 Precision

Statistical parameters are expressed in accordance with ISO 5725[1].

8.1 Repeatability

$r = 33{,}4;\qquad s_r = 29{,}4$

where

r is the repeatability limit;

s_r is the standard deviation of repeatability.

8.2 Reproducibility

$R = 41{,}0;\qquad s_R = 35{,}8$

where

R is the reproducibility limit;

s_R is the standard deviation of reproducibility.

9 Test report

The test report shall specify

- the method used,
- the test result obtained, and
- if the repeatability has been checked, the final quoted result obtained.

It shall also mention all operating details not specified in this part of ISO 8128, or regarded as optional, together with details of any incidents which may have influenced the test result.

The test report shall include all information necessary for the complete identification of the sample.

---

1) HMF = 5-hydroxymethylfurfural

Dimensions in centimetres

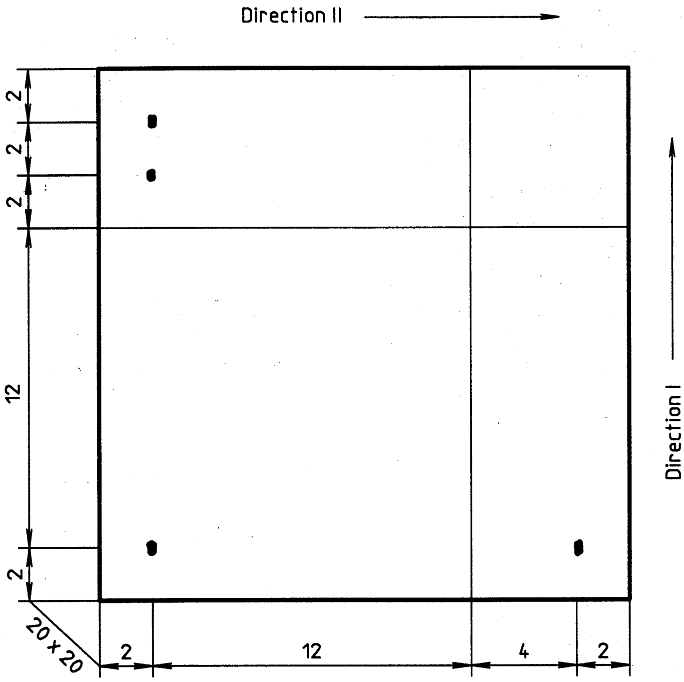

Figure 1 — Two-directional thin-layer chromatography

Annex A
(informative)

Bibliography

[1] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.*

[2] ISO 8128-1:1993, *Apple juice, apple juice concentrates and drinks containing apple juice — Determination of patulin content — Part 1: Method using high-performance liquid chromatography.*

[3] *Official Methods of Analysis,* Vol. 12, Washington DC: AOAC, 1975, p. 463.

[4] LINDROTH, S. and NISKANWN, A. *J. Food Sci.,* **43**, 446-448 (1978).

©ESA

የኢትዮጵያ የደረጃዎች ኤጀንሲ

Ethiopian Standards Agency

Organization and Objectives

The Ethiopian Standards Agency (ESA) is the national standards body of Ethiopia established in 2010 based on regulation No. 193/2010.ESA is established due to the restructuring of Quality and Standards Authority of Ethiopia (QSAE) which was established in 1970.

ESA's objectives are:-

- Develop Ethiopian standards and establish a system that enable to check weather goods and services are in compliance with the required standards,
- Facilitate the country's technology transfer through the use of standards,
- Develop national standards for local products and services so as to make them competitive in the international market.

Ethiopian Standards

The Ethiopian Standards are developed by national technical committees which are composed of different stakeholders consisting of educational Institutions, research institutes, government organizations, certification, inspection, and testing organizations, regulatory bodies, consumer association etc. The requirements and/ or recommendations contained in Ethiopian Standards are consensus based that reflects the interest of the TC representatives and also of comments received from the public and other sources. Ethiopian Standards are approved by the National Standardization Council and are kept under continuous review after publication and updated regularly to take account of latest scientific and technological changes. Orders for all Ethiopian Standards, International Standard and ASTM standards, including electronic versions, should be addressed to the Documentation and Publication Team at the Head office and Branch (Liaisons) offices. A catalogue of Ethiopian Standards is also available freely and can be accessed in from our website.

ESA has the copyright of all its publications. No part of these publications may be reproduced in any form without the prior permission in writing of ESA.

International Involvement

ESA, representing Ethiopia, is a member of the International Organization for Standardization (ISO), and Codex Alimentarius Commission (CODEX). It also maintains close working relations with the international Electro-technical Commission (IEC) and American Society for Testing and Materials (ASTM).It is a founding member of the African Regional Organization for standardization (ARSO).

More Information?
Contact us at the following address.
The Head Office of ESA is at Addis Ababa.

☎ 011- 646 06 85, 011- 646 05 65
📠 011-646 08 80
✉ 2310 Addis Ababa, Ethiopia
E-mail: info@ethiostandards.org,
Website: www.ethiostandards.org

Standard Mark

DRAFT